


AUTHOR QUERY FORM

 ELSEVIER	Journal: TRPLSC Article Number: 1182	Please e-mail or fax your responses and any corrections to: E-mail: corrections.esnl@elsevier.thomsondigital.com Fax: + 31 20 485 2521
---	---	---

Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list. Note: if you opt to annotate the file with software other than Adobe Reader then please also highlight the appropriate place in the PDF file. To ensure fast publication of your paper please return your corrections within 48 hours.

For correction or revision of any artwork, please consult <http://www.elsevier.com/artworkinstructions>.

Please note that the figure resolution in these proofs is lower than the original figures to avoid problems with emailing large file sizes. The figure resolution in the final publication will be equivalent to the original figure submission. Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Click on the 'Q' link to go to the location in the proof.

Location in article	Query / Remark: click on the Q link to go Please insert your reply or correction at the corresponding line in the proof
Q1 Q2 Q3 Q4	<p>Please confirm that given names and surnames have been identified correctly.</p> <p>Acronyms in author affiliations given in full at first mention, please check OK.</p> <p>Please check the citation of Box 1. if not appropriate, Please cite it in appropriate place.</p> <p>Ref. 14 details added, please check OK.</p> <div data-bbox="367 1293 894 1357"> Please check this box or indicate your approval if you have no corrections to make to the PDF file </div> <div data-bbox="938 1293 1003 1357"> <input type="checkbox"/> </div>

Thank you for your assistance.

Update on the genomics and basic biology of *Brachypodium*

International *Brachypodium* Initiative (IBI)

Pilar Catalan^{1,2*}, Boulos Chalhou³, Vincent Chochois⁴, David F. Garvin⁵,
 Q1 Bert Hasterok⁶, Antonio J. Manzaneda⁷, Luis A.J. Mur⁸, Nicola Pecchioni⁹,
 Søren K. Rasmussen¹⁰, John P. Vogel¹¹, and Aline Voxeur^{12,13}

¹ Department of Agricultural and Environmental Sciences, High Polytechnic School of Huesca, University of Zaragoza, Carretera Cuarte km 1, 22071 Huesca, Spain

Q2 ² Department of Botany, Institute of Biology, Tomsk State University, Lenin Avenue 36, Tomsk 634050, Russia
³ Unité de Recherche en Génomique Végétale (URGV), Institut National de la Santé et de la Recherche Médicale (INRA)–Centre National de la Recherche Scientifique (CNRS)–Université Evry-Val d'Essonne (UEVE), Organization and Evolution of Plant Genomes (OEPG), 91057 Evry CEDEX, France

⁴ Commonwealth Scientific and Industrial Research Organisation (CSIRO) Plant Industry, Black mountain laboratories, Clunies Ross Street, 2601 Acton, Canberra, Australia

⁵ United States Department of Agriculture (USDA)–Agricultural Research Service (ARS) Plant Science Research Unit, 411 Borlaug Hall, University of Minnesota, 1991 Upper Buford Circle, St Paul, MN 55108, USA

⁶ Department of Plant Anatomy and Cytology, University of Silesia in Katowice, Jagiellonska 28, 40-032 Katowice, Poland

⁷ Departamento Biología Animal, Biología Vegetal y Ecología, Universidad de Jaén, Paraje las Lagunillas s/n, 23071 Jaén, Spain

⁸ Aberystwyth University, Institute of Biological, Environmental and Rural Science, Aberystwyth, SY23 3DA, Wales, UK

⁹ Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola 2, 42122 Reggio Emilia, Italy

¹⁰ Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark

¹¹ USDA–ARS Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710, USA

¹² Institut National de la Recherche Agronomique (INRA), Institut Jean-Pierre Bourgin (IJPB) Unité Mixte de Recherche (UMR) 1318, Saclay Plant Science, 78000 Versailles, France

¹³ AgroParisTech, Institut Jean-Pierre Bourgin (IJPB) UMR1318, Saclay Plant Science, 78000 Versailles, France

The scientific presentations at the First International *Brachypodium* Conference (abstracts available at <http://www.brachy2013.unimore.it>) are evidence of the widespread adoption of *Brachypodium distachyon* as a model system. Furthermore, the wide range of topics presented (genome evolution, roots, abiotic and biotic stress, comparative genomics, natural diversity, and cell walls) demonstrates that the *Brachypodium* research community has achieved a critical mass of tools and has transitioned from resource development to addressing biological questions, particularly those unique to grasses.

A model for grass genome organization

This report highlights recent advances made in *Brachypodium* research, focusing on the use of *B. distachyon* and related species to understand biological processes. Its experimental and genomic tractability allow *B. distachyon* to act as a functional genomic test-bed to accelerate the improvement of grain, forage, and biomass crops. Its strengths as a model plant (e.g., short generation time, efficient *Agrobacterium*-mediated transformation, and availability of mutant collections) are described in [1] and other reviews published in the past 5 years. The main web portal for

Brachypodium (<http://www.brachypodium.org>) contains a genome browser and links to community resources. In addition, two project-specific websites (<http://brachypodium.pw.usda.gov/> and <http://www-urgv.versailles.inra.fr/tilling/brachypodium.htm>) provide access to T-DNA and Tilling resources, respectively.

The compact nature of the fully sequenced [2] *Brachypodium distachyon* genome is a major reason for the success of *B. distachyon* as a model system, and provides unique opportunities to study various aspects of grass genome organization and evolution. Moreover, as a monocot reference, it permits comparisons of genomic landscape dynamics with the dicot model *Arabidopsis thaliana*. Thus, *B. distachyon* has become an appealing target for plant molecular cytogenetics.

One of the most informative cytological tools is chromosome painting (CP), which enables unique and unambiguous visualization of individual chromosomes or large segments, both during cell division and even at interphase, using fluorescence *in situ* hybridization with specific DNA probes. CP was initially applied to vertebrate systems. Whole-genome sequencing (WGS) and large-insert genomic DNA libraries allow its application to small-genome plants such as *A. thaliana*. The sequencing of the *B. distachyon* genome [2] combined with its low (5) chromosome number and a well-developed cytogenetic infrastructure has allowed the CP of several *Brachypodium* species [3], a pioneering application of CP in monocots.

The chromosomes of *B. distachyon* can be selectively painted to address important questions about grass genome

Corresponding author: Pecchioni, N. (nicola.pecchioni@unimore.it).

Keywords: *Brachypodium*; genomics; model plant; crop improvement.

*Contributors in alphabetic order.

1360-1385/

© 2014 Published by Elsevier Ltd. <http://dx.doi.org/10.1016/j.tplants.2014.05.002>

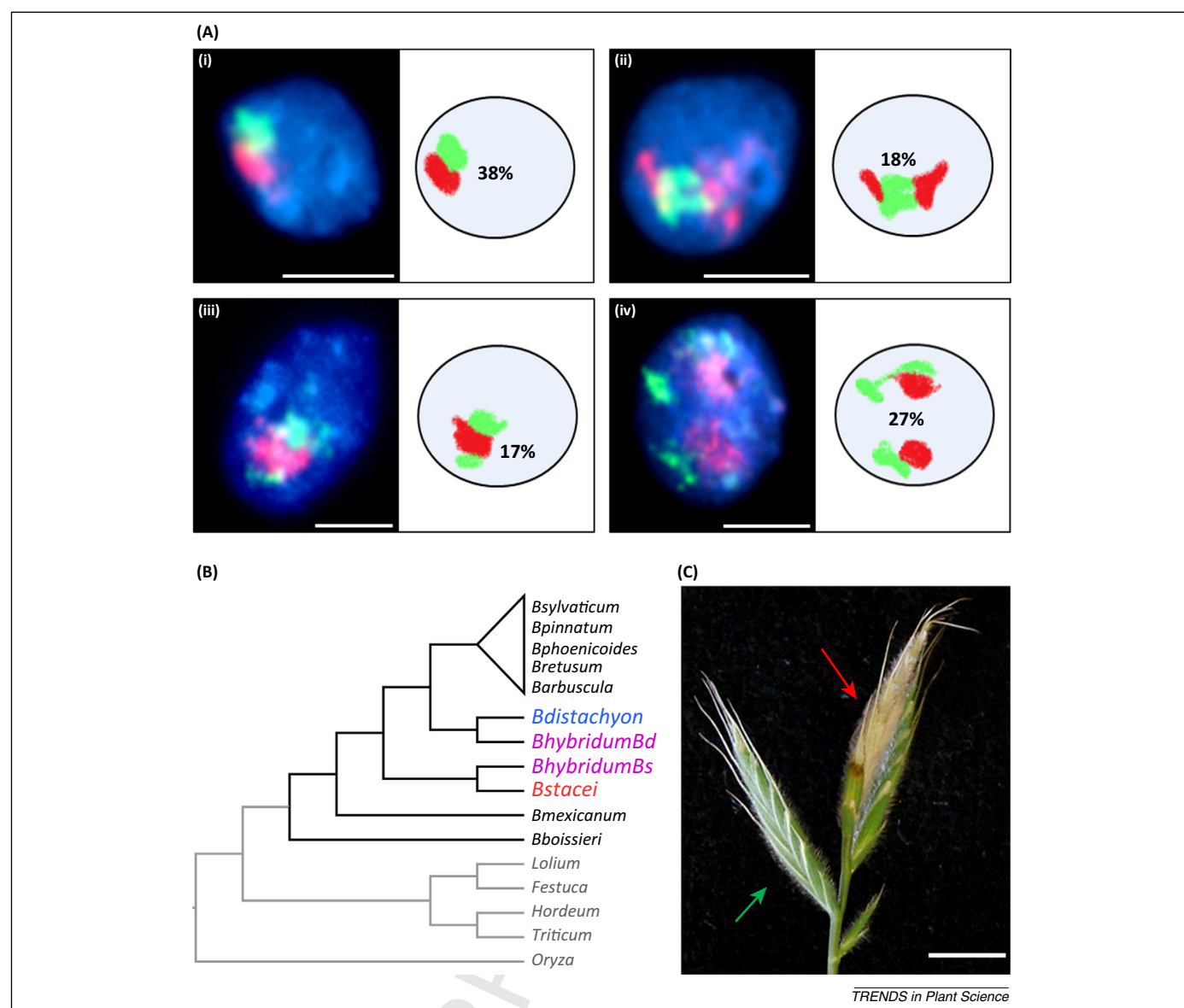


Figure 1. (A) Different arrangements of the Bd2 homologous chromosome territories (CTs) and the observed frequencies (%) of their occurrence in interphase nuclei of roots of *B. distachyon*: (i) complete association; (ii) top (green fluorescence) and (iii) bottom (red fluorescence) arm-only association; (iv) complete separation. Chromatin stained with DAPI (4',6-diamidino-2-phenylindole; blue fluorescence). Scale bars: 5 μ m. For better visualization the respective arrangements of CTs have been schematically shown on the diagrams next to photomicrographs. Documentation courtesy of Ewa Breda (University of Silesia in Katowice, Poland). (B) Evolution of the three *B. distachyon*-complex species. Summarized low-copy nuclear GIGANTEA (G1) gene tree showing the phylogenetic reconstruction of *B. stacei*, *B. distachyon*, and *B. hybridum* [*B. hybridum* shows G1 copies from both stacei-type (BsBs genome) and distachyon-type (BdBd genome) parents, coinherited from bidirectional crosses]. (C) *Fusarium* head blight symptoms on Bd21 spikes following point inoculation (red arrow) with *Fusarium graminearum* strain FgUK1 at 6 days after inoculation. An asymptomatic spike (green arrow) is shown for comparison. Scale bar, 1 cm. Photograph courtesy of Paul Nicholson and Antoine Peraldi (John Innes Centre, UK).

structure and evolution (in Figure 1 we demonstrate how they are arranged at interphase). CP of *B. distachyon* chromosome 2 (Bd2) in the nuclei of root cells revealed that Bd2 homologous chromosome territories can assume four different configurations that are observed at different frequencies (Figure 1A). This is one example where research in *Brachypodium* could lead the way in determining whether and how nuclear structure is linked to cell differentiation and tissue-specific gene expression.

A tractable model for inter- and intraspecific diversity

The genus *Brachypodium* contains 15–18 species with unusually variable chromosome numbers and ploidy levels. This diversity was a subject of interest long before *B. distachyon* became a model grass. WGS of *B. distachyon*

[2], together with the advent of inexpensive next-generation sequencing (NGS) technologies, set the stage for high-resolution investigation of the genomic diversity and evolutionary relationships in the genus.

It was recently demonstrated that '*B. distachyon*' is a complex of three separate species: two diploids (*B. distachyon*, *B. stacei*) and their derived allotetraploid (*B. hybridum*) ([4] and Figure 1B). The genomes of *B. stacei* and *B. hybridum* are being sequenced, and gene expression is being compared between all three species, to serve as a model for speciation through adaptation and polyploidization. To develop further this trio of species as a model for plant polyploidy, allopolyploids are being developed through interspecific hybridization between *B. distachyon* and *B. stacei* with the aim of reproducing *B. hybridum*

(Vinh Ha Dinh Thi and B.C., unpublished). This system offers experimental advantages (compact genomes with little repetitive DNA, small and easily grown plants that can be transformed efficiently) that will facilitate investigating the role of polyploidy in speciation and adaptation, a topic of great interest to cereal breeders.

The analysis of intraspecific diversity in *B. distachyon* is also underway through NGS resequencing of 54 diverse natural accessions. High polymorphism rates of up to one single-nucleotide polymorphism (SNP) per 200 bp have been detected (J.P.V., unpublished). Because the *B. distachyon* accessions display considerable phenotypic variation in a plethora of economically important traits (e.g. seed size, biomass, cell wall composition, flowering time), knowledge of genome sequence variation will facilitate genome-wide association mapping and positional cloning of economically important genes. In addition, several mutant populations have been established [1] that will greatly increase the utility of these new sequence resources.

A model for abiotic stress

B. distachyon, *B. stacei*, and *B. hybridum* all grow in a wide range of habitats under marked environmental gradients. Distinct genotypes are thus subject to different abiotic stresses which might have exerted, from speciation until present, different selective pressures on stress tolerance-related traits.

Detection of adaptive variation of stress-tolerance traits in response to abiotic conditions requires the following: (i) significant genetic variation in the trait of interest, (ii) a match between adaptive genetic variation and environmental variation (e.g., local adaptation across the gradient), and (iii) positive selection for these traits in genotypes growing under abiotic stress. Progress has been made in screening for natural variation in stress tolerance among *B. distachyon* accessions (reviewed in [1]), the first step towards determining the heritability of adaptive traits. However, full understanding of the adaptive significance of tolerance trait variation awaits experimental evaluation of the effects of such variation on fitness in natural populations.

Progress toward understanding abiotic stress adaptation at a molecular level is being made. Promising results come from the recent characterization of a microRNA (miRNA) network controlling cell division during stress, part of a search for epigenomic regulatory mechanisms underlying drought stress [5]. Further, evidence of ancient adaptive evolution of temperate Pooideae species was inferred from nucleotide substitution rates and signatures of positive selection in genes induced by low temperature [6]. Finally, adapted genotypes of *B. distachyon* and *B. hybridum* may reveal how genomic changes such as whole-genome duplication influence ecological tolerances to abiotic stress. In fact, differential tolerance to water stress between *B. distachyon* and *B. hybridum* seems to drive the ecogeographical differentiation of these species [7].

A model pathosystem for multiple cereal diseases

Pests and pathogens are major contributors to global food insecurity. Shifting climate patterns are altering disease ranges and facilitating the emergence of virulent strains.

Thus, we need a better understanding of plant–pathogen interactions to develop rapidly new and preferably durable sources of disease resistance. *B. distachyon* has emerged as a powerful tool to elucidate defense responses in the Poaceae. A major advance has been the demonstration that *B. distachyon* serves as a host for many pathogens that cause diseases such as rice blast, *Fusarium* head blight (FHB; Figure 1C), and barley stripe mosaic virus (BSMV) (reviewed by [4]). Studies are now exploiting the genetic and functional genomic resources available for *B. distachyon* to elucidate host responses to pathogens. An elegant example is the characterization of *B. distachyon* UDP-glycosyltransferases that can detoxify the mycotoxin deoxynivalenol produced by *Fusarium graminearum*, the casual pathogen of FHB [8]. As such studies progress they may identify commonalities in host responses to pathogens which could represent key defense nodes that are potential sources of durable resistance to many pathogens. Translation of this knowledge into improved crop varieties will involve identifying orthologous genes or linked molecular markers in crop germplasm. Such a strategy contributed to the targeting of *Pch1* eyespot resistance in wheat (*Triticum aestivum*) [9]. However, the absence of an ortholog of the wheat *Lr34* leaf rust resistance gene in *B. distachyon* [10] indicates that successful transfer of information between *B. distachyon* and grass crops is not guaranteed. Another powerful means of increasing crop resistance is the direct transfer of genes from *B. distachyon* into elite cereal germplasm through transformation. The success of this approach is enhanced by the close relationship between *B. distachyon* and the cereals.

A model for the grass cell wall and biomass accumulation

Similarly to other grasses, *B. distachyon* has a type II wall that differs markedly from the type I walls found in dicots. Until now, few studies have focused on characterizing *B. distachyon* cell wall polysaccharides and their biosynthetic enzymes. Detailed biochemical characterization of these polysaccharides, their distribution in different tissues and organs, and their roles during development need to be investigated. Characterization of mixed linkage glucans (a polymer unique to grasses) in *B. distachyon* seeds showed surprising enrichment in (1→3) linkages and that arabinoxylans were more substituted compared to wheat, barley (*Hordeum vulgare*) or oat (*Avena sativa*).

In comparison with polysaccharides, *B. distachyon* lignin has been studied in greater detail by genetic and biochemical characterization of some of the enzymes required for lignin biosynthesis. The enzymes are encoded by gene families, but each gene has a distinct function, and are thus good targets for mutagenesis or introgression [11]. As an elegant example of the potential of the model grass, mutation of the cinnamyl alcohol dehydrogenase (CAD1) gene has been shown to lead to a 25% decrease in lignin content, resulting in improved saccharification [12]. These results, taken together with the possibility to increase biomass by modifying polysaccharide-related metabolism, demonstrate that *B. distachyon* is an excellent model for identifying genes important for developing biomass crops with improved conversion into bioenergy or new materials (Box 1).



Box 1. *Brachypodium distachyon* root and rhizosphere for underground discoveries

Plant roots provide a multitude of essential functions like mechanical support, water and nutrient uptake, defense against soil pathogens and toxins. Compared to shoots, roots have been understudied, and offer important opportunities to increase global food production, and save water, land, and fossil fuels. Most molecular root research has been conducted on *Arabidopsis*, but its dicotyledonous root system has a different morphology, architecture, anatomy, and biochemistry from cereals such as wheat or barley. Thus, *B. distachyon* now has many of the tools available for *A. thaliana* but a root system similar to that of temperate cereals (Figure 1).

B. distachyon root system is composed of three root types (Figure 1). A single primary seminal root (PSR) emerges at germination; this is followed approximately 2 weeks later by one or two coleoptile nodal roots (CNR) from the coleoptile node located on the mesocotyl, about half way between the seed and the leaf nodes, and finally, 3–4 weeks after germination, leaf nodal roots (LNR) start to emerge from the leaf nodes [13]. In *B. distachyon* the number of nodal roots, but not the

seminal primary root, varies genetically and in response to water, opening the possibility of selecting root systems for specific soil conditions (V.C., unpublished). *B. distachyon* root variation can be studied in much smaller volumes of soil than maize or rice, permitting the characterization of the role of mature root systems during flowering and seed development [13]. Flowering and seed development are highly susceptible to drought, and knowledge of root genes at these stages can be applied to crop improvement through marker-assisted breeding.

Since the emergence of *B. distachyon* as a molecular model it has been applied in several fields relevant to roots, including root system architecture of cereals, response to biotic (pathogens such as *Fusarium*, *Rhizoctonia*) and abiotic (nutrient levels, drought) stresses, auxin homeostasis [14], and symbiotic root–microbe interactions such as arbuscular mycorrhizal fungi [15]. *B. distachyon* is an exciting new model for root research, opening the way to understanding monocotyledon root biology, and eventually leading to the improvement of major temperate crops.

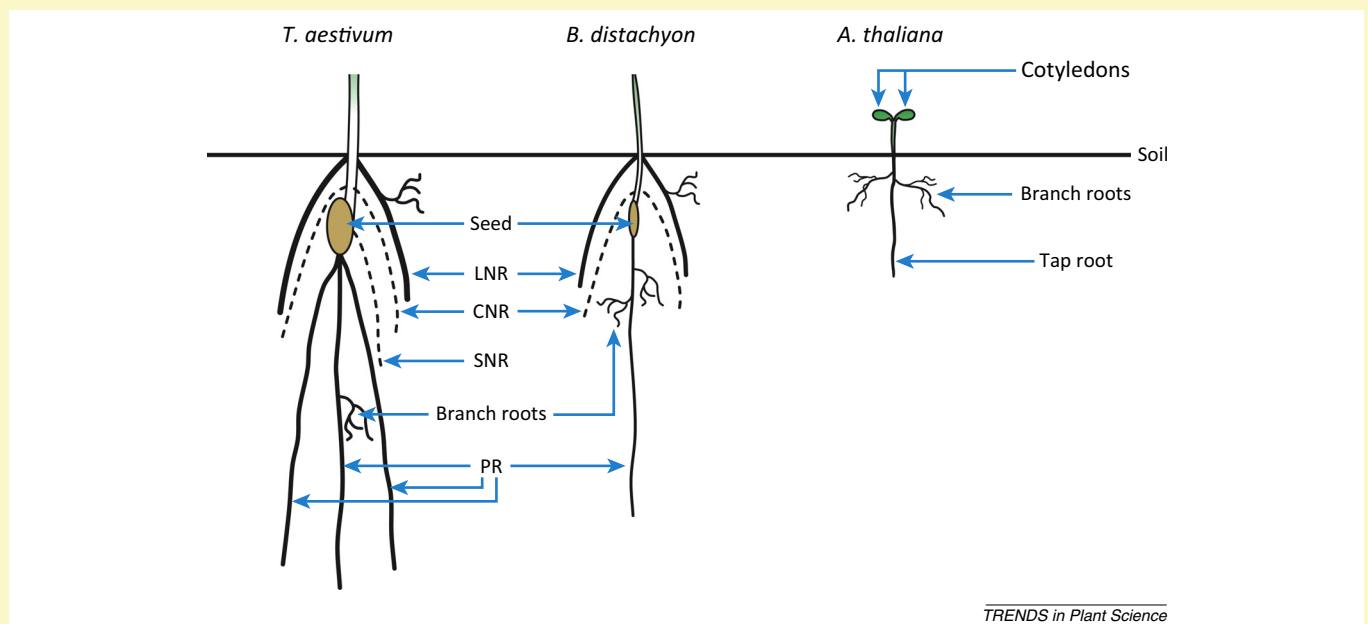


Figure 1. Comparison of wheat (*T. aestivum*), *Brachypodium distachyon*, and *A. thaliana* root systems. Horizontal line, soil level. Abbreviations: CNR, coleoptile node axile root; LNR, leaf node axile root; PR, primary axile root; SNR, scutellar node axile root.

Taken together, these advances demonstrate the wide applicability of *Brachypodium* as a model system and underscore the maturity of the system.

Acknowledgements

We thank the US Department of Energy Joint Genome Institute for sequencing through the Community Science Program. Authors acknowledge the following contributions. P.C., Spanish Ministry of Economy and Competitiveness (CGL2012-39953-C02-01); V.C., Australian Grain Research and Development Corporation (CSP00129); D.G., USDA (CRIS 3640-21000-027); R.H., Polish National Science Centre (DEC-2012/04/A/NZ3/00572); A.M., European Commission, Marie Curie Action (PIOF-GA-2008-220983), Spanish Ministry of Economy and Competitiveness (RYC-2010-06237, CGL2012-30838); J.V., Office of Science (BER), US Department of Energy, USDA (CRIS 5325-21000-017). We apologize to all colleagues whose papers were not cited owing to space limitations.

References

- Mur, L.A. *et al.* (2011) Exploiting the *Brachypodium* Tool Box in cereal and grass research. *New Phytol.* 191, 334–347

- International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature* 463, 763–768
- Idziak, D. *et al.* (2011) Painting the chromosomes of *Brachypodium*: current status and future prospects. *Chromosoma* 120, 469–479
- Catalan, P. *et al.* (2012) Evolution and taxonomic split of the model grass *Brachypodium distachyon*. *Ann. Bot.* 109, 385–405
- Bertolini, E. *et al.* (2013) Addressing the role of microRNAs in reprogramming leaf growth during drought stress in *Brachypodium distachyon*. *Mol. Plant* 6, 423–443
- Vigeland, M.D. *et al.* (2013) Evidence for adaptive evolution of low-temperature stress response genes in a Poideae grass ancestor. *New Phytol.* 199, 1060–1068
- Manzaneda, A.J. *et al.* (2012) Environmental aridity is associated with cytotype segregation and polyploidy occurrence in *Brachypodium distachyon* (Poaceae). *New Phytol.* 193, 797–805
- Schweiger, W. *et al.* (2013) Functional characterization of two clusters of *Brachypodium distachyon* UDP-glycosyltransferases encoding putative deoxynivalenol detoxification genes. *Mol. Plant Microbe Interact.* 26, 781–792
- Burt, C. and Nicholson, P. (2011) Exploiting co-linearity among grass species to map the *Aegilops ventricosa*-derived *Pch1* eyespot resistance

in wheat and establish its relationship to *Pch2*. *Theor. Appl. Genet.* 123, 1387–1400

10 Krattinger, S.G. *et al.* (2011) Lr34 multi-pathogen resistance ABC transporter: molecular analysis of homoeologous and orthologous genes in hexaploid wheat and other grass species. *Plant J.* 65, 392–403

11 Bukh, C. *et al.* (2012) Phylogeny and enzyme activity of the cinnamyl alcohol dehydrogenase gene family in *Brachypodium distachyon*. *J. Exp. Bot.* 63, 6223–6236

12 Bouvier d'Yvoire, M. *et al.* (2013) Disrupting the cinnamyl alcohol dehydrogenase 1 gene (BdCAD1) leads to altered lignification and improved saccharification in *Brachypodium distachyon*. *Plant J.* 73, 496–508

13 Watt, M. *et al.* (2009) The shoot and root growth of *Brachypodium* and its potential as a model for wheat and other cereal crops. *Funct. Plant Biol.* 36, 960–969

14 Pacheco-Villalobos, D. *et al.* (2013) Disturbed local auxin homeostasis enhances cellular anisotropy and reveals alternative wiring of auxin–ethylene crosstalk in *Brachypodium distachyon* seminal roots. *PLoS Genet.* 9, e1003564

15 Hong, J.J. *et al.* (2012) Diversity of morphology and function in arbuscular mycorrhizal symbioses in *Brachypodium distachyon*. *Planta* 236, 851–865

501

502

503

504

505

506

07

08

509

510

511

512



04

07

08

509

510

511

512